Mixed Segregation of Chromosomes During Single-Division Meiosis of *Saccharomyces cerevisiae*

Gil Sharon[1] and Giora Simchen

Department of Genetics, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Manuscript received August 20, 1989
Accepted for publication March 9, 1990

ABSTRACT

Normal meiosis consists of two consecutive cell divisions in which all the chromosomes behave in a concerted manner. Yeast cells homozygous for the mutation *cdc5*, however, may be directed through a single meiotic division of a novel type. Dyad analysis of a *cdc5/cdc5* strain with centromere-linked markers on four different chromosomes has shown that, in these meioses, some chromosomes within a given cell segregate reductionally whereas others segregate equationally. The choice between the two types of segregation in these meioses is made individually by each chromosome pair. Different chromosome pairs exhibit different segregation tendencies. Similar results were obtained for cells homozygous for *cdc14*.

MEIOSIS in eukaryotes consists of two consecutive nuclear divisions. In the first division centromeres segregate reductionally and in the second divide equationally. Diploid sporulating cells of *Saccharomyces cerevisiae* undergo a meiotic process, which is similar to that of most other eucaryotic organisms. Usually, the products of meiosis in yeast are four haploid spores, a tetrad, held together in an ascus. However, certain mutations result in aberrant forms of meiosis, which give rise to unusual spores. When diploid strains homozygous for either of the temperature-sensitive mutations *cdc5* or *cdc14* are shifted to the restrictive temperature after initiating the first meiotic division, they form dyads (two-spored asci) rather than tetrads (SCHILD and BYERS 1980). Each dyad contains two viable diploid spores. Genetic analysis of such spores has shown that they result from a single division in which centromeres have gone through a first-division segregation (SCHILD and BYERS 1980). Strains homozygous for *spo12* and/or *spo13* also tend to form diploid dyads, but in these cases each ascus usually appears to have resulted from a single division in which the centromeres have undergone a second-division segregation (KLAPHOLZ and ESPOSITO 1980). It seems, therefore, that a successful equational division is not essential for spore formation and maturation, nor is it necessary to have a successful reductional division prior to the equational one. In all of these mutants, meiotic events known to precede the divisions, such as formation of synaptonemal complexes and meiotic recombination, have been observed.

During meiosis, a temperature shift from 23° to 34° can cause an arrest of *cdc5/cdc5* diploids prior to either the first or the second division, depending on the time of the shift. If the shift is made at the beginning of meiosis, arrested cells are mononucleate. Ultrastructural analysis of these cells has shown (SCHILD and BYERS 1980) that they have two spindle poles, situated on opposite sides of the nucleus. The microtubules from the poles are not interconnected, but extend into the nucleus. If the shift is made after the cells have already gone through the first meiotic division they do not go through the second one. Two spindles which are unable to elongate are observed, and the four spindle poles encapsulate into two viable diploid spores (SCHILD and BYERS 1980). We wanted to investigate the effects of a late release of *cdc5* homozygotes arrested at the mononucleate stage. Our initial attempt was to test whether cells, under these conditions, could advance to the second meiotic division without going through the first one. Somewhat unexpectedly we found that although diploid dyads were produced, their chromosome segregation pattern was neither that of the first meiotic division nor that of the second. Genetic analysis of colonies produced by these spores, using markers linked to four centromeres, has shown that in most cases, some of the centromeres within a single sporulating cell have segregated reductionally whereas the others have segregated equationally. The apparent independence in segregation of individual chromosomes demonstrates that the decision as to how to segregate is made at a subcellular level. Significant differences in the tendency to segregate either reductionally or equationally were detected among the four chromosomes that were examined. These differences imply that the choice between the two types of segregation is made inde-

[1] Present address: Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

pendently at the level of each chromosome pair. Similar "mixed" segregations were also observed in a strain homozygous for the mutation *cdc14*.

MATERIALS AND METHODS

Yeast strains:

Strain 11: *MAT***a**, *cdc5–1, ura3, can1–11, ade1, leu1, met14*.
Strain 22: *MATα, cdc5–1, cdc7, ura3, lys2*.
Strain 33: *MATα, cdc14–3, ade1, trp1, his2, leu1, met14*.
Strain 44: *MAT***a**, *cdc14–3, ura3, can1–11*.

These strains were derived from crosses among laboratory strains of various origins.

Strain 1122 is a diploid constructed by mating strain 11 with strain 22.

Strain 1122-lU was constructed by integrative transformation (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981) of strain 1122 with the plasmid pJM82. In order to direct the integration into the desired location, the plasmid was cleaved by *Hin*dIII within the chromosomal insertion. The integration placed the external *URA3* gene as well as pBR322 sequences close to *CEN7*, on the right arm of chromosome *VII* which carried the allele *leu1*. As a result, *URA3* and *LEU1* in this strain were situated *in trans*, on opposite arms of chromosomes *VII*. Strain 3344 is a diploid constructed by mating strain 33 with strain 44.

Plasmids: The plasmid pJM82 was obtained from JEANNE P. MARGOLSKEE. It consists of the plasmid YIp5 (SCHERER and DAVIS 1979) with an insertion of a 1.5-kb, *Bam*HI-*Eco*RI DNA fragment from the right arm of chromosome *VII*, 34 kb from *CEN7* (J. P. MARGOLSKEE, personal communication).

Media: Liquid culture media PSP2 and SPM were prepared according to SIMCHEN, PINON and SALTS (1972). MIN (minimal solid medium) is a minimal glucose medium. Complete solid synthetic medium was prepared in the same way but included adenine and uracil, as well as amino acids, as described previously (SHILO, SIMCHEN and SHILO 1978). Selective media did not include the component selected for. Canavanine solid medium has been described (KASSIR and SIMCHEN 1985). SPO solid sporulation medium has been described (HICKS and HERSKOWITZ 1976).

Shift-down experiments: Cells were transferred to liquid sporulation medium (SPM) after reaching a titer of 10^7 cells/ml in presporulation medium (PSP2) at 23°. Half an hour after the transfer to SPM, the cells were shifted to the restrictive temperature (34°), thus allowing "late" cells to complete the mitotic cycle before imposing the temperature regime. At different times, subcultures were shifted down to the permissive temperature (23°) and cells were allowed to continue sporulation under permissive conditions. The frequencies of tetrads, dyads and monads (one-spored asci) were scored 60 hr after transfer to sporulation medium.

Scoring of temperature-sensitive alleles: Three cell-division cycle genes with temperature-sensitive alleles were used and scored in these experiments, namely *cdc5*, *cdc14*, and *cdc7*. The temperature-sensitive phenotype was scored by replica plating on prewarmed YEPD plates followed by incubation at 37° for 2 days. When temperature-sensitive alleles at two different loci were examined in the same set of progeny, for instance both *cdc5* and *cdc7*, segregation was scored by mating to testers carrying only one of the *ts* mutations, followed by replication of the resulting diploids onto YEPD at 37°.

Spore analysis: Tetrads or dyads were dissected as described by DAVIDOW, GOETSH and BYERS (1980). Each dyad was placed separately next to a droplet of full-strength Glusulase (Endo Labs) and following wall lysis, the spores were planted on dissection agar. This procedure ensured that the spore colonies were the products of genuine sister spores. The germinating colonies were plated on SPO plates and their ability to form spores was examined after incubation at 23° for a week. Mating ability was tested by crossing with **a** and α auxotrophic tester strains. The genotypes of nonmater, sporulating colonies were examined further by progeny testing. In most cases, the progeny testing consisted of random spore analysis of the resporulated asci, a proce-

TABLE 1

Frequencies of asci of *cdc5/cdc5* strain following temperature shift-down at various times during arrested sporulation

Shift-down time (h)	Unsporulated cells (%)	Monads (%)	Dyads (%)	Triads + tetrads (%)
A				
0	30.5	4.5	10.5	54.5
→17	55.0	10.0	17.0	18.0
22	65.0	15.0	11.0	9.0
→25	75.5	16.5	6.0	2.0
31	88.5	8.5	2.0	1.0
→34	84.0	9.5	6.0	0.5
36	86.0	9.0	4.0	1.0
→39	85.0	8.5	6.5	0.0
60	88.5	8.5	2.5	0.5
B				
12	41.0	14.5	19	25.5
15	55.5	14.0	23	7.5
→18	82.0	8.5	8	1.5
24	90.5	6.0	3	0.5
32	90.5	7.5	2	0.0

Sporulation was initiated by the transfer of diploid cells of strain 1122 from PSP2 to SPM media at 23°. Half an hour later, the temperature was shifted to 34°. At various times after the initiation of sporulation, aliquots were shifted down to 23°. Asci frequencies in the aliquots were scored 60 h after the initiation. A and B are two independent experiments. Monads, dyads, triads and tetrads are one-spored, two-spored, three-spored and four-spored asci, respectively (sample size 200–300 cells). →, Shift-down times from which genotypes of dyads and spores were analyzed.

TABLE 2

Spore viability in dyads of strain 1122-lU (shift-down at 18 hr)

	No viable	One viable	Two viable
Both spores are **a**/α	0	0	97 (89 were analyzed)
One of the spores is **a**/α	0	109 (105 were analyzed)	48[a] (all were analyzed)
Others[b]	618	701	469
Total 2042	618	810	614

[a] The 48 dyads in which only one of the spores was a diploid **a**/α consisted of 27 in which the other spore was either **a** or α, and 21 in which the other spore was unidentified (a nonmater that could not sporulate).

[b] This category consists of dyads of which both spores did not germinate and therefore could not be characterized, and dyads which could not be analyzed because none of the spores gave rise to sporulating **a**/α diploids. This latter group consisted of maters (**a**s or αs) and of nonmaters which had not undergone sporulation for unidentified reasons (of 538 spores from dyads having two viable spores, 19 were nonmaters but could not sporulate).

dure which is fast and simple but can be somewhat imprecise. In cases where a precise determination was required, we identified the genotype of the colonies by progeny-tetrad analysis as well. The results obtained by both methods were in complete agreement. Random spore analysis was performed as follows. Ascus walls were digested for three hours at 37° with Glusulase diluted 1/10 in water. The asci were then sonicated with Branson Sonifier microtip at level 7 for approximately 40 sec (six doses of 7 sec each). The spores were plated on YEPD, incubated for 5 days at 23° and the resulting colonies were analyzed by replica plating.

Adjustments for comparison analysis: Segregation of markers among dyads having a single viable spore was determined by analyzing one spore per dyad, whereas in dyads having two viable spores, segregation was determined by analysis of both spores. In order to compare the distribution of genotypes among dyads with only one viable spore with dyads containing two viable spores, an adjustment had to be made. The one-viable spore dyads were scored separately and each was given half a weight in the analysis, compared to dyads with two viable spores. In dyads where gene conversion of a given marker took place, the segregation of the marker in one spore appeared to be different from its segregation in the other. Such markers were therefore scored as two half-weight cases, each contributing to the overall size of a different category of segregants. Another adjustment had to be made in cases where one of the two viable spores of a dyad was an **a**/α and the other was either an **a** or an α mater. In these cases, only the **a**/α spore was analyzed and given half a weight in the analysis.

RESULTS

Analysis of diploid dyads (two-spored asci): Cells of strain 1122-1U, homozygous for *cdc5*, were shifted down from restrictive to permissive temperature at various times after the onset of meiosis (see MATERIALS AND METHODS for details). A relatively high proportion of dyads was observed in subcultures of intermediate shift-down times (12–22 hr, see Table 1 for results obtained in two independent experiments). At later shift-down times, there was a decline in the frequency of dyads but it was less pronounced than the decline in the frequency of tetrads. Preliminary experiments (not shown) demonstrated that some of the dyads, especially ones which belong to early shift-down subcultures were haploid-spored. In order to minimize the proportion of these dyads among the ones examined, we analyzed dyad populations from intermediate and late shift-down subcultures (shifted at 17, 18, 25, 34 and 39 hr after the transfer to SPM medium, see Table 1). All these populations gave similar results. Observations from the most thoroughly studied population (shifted-down 18 hr after transfer to SPM, in experiment B listed in Table 1) are described below:

Of the 2042 dyads isolated and dissected (see MATERIALS AND METHODS), 614 had two viable spores, 810 had one viable spore (the other spore did not give rise to a colony) and 618 had two inviable spores (Table 2). **a**/α diploid spores (which gave rise to nonmater colonies with sporulation ability) were further analyzed by resporulation of these colonies and progeny-testing, in order to determine their genotypes (see MATERIALS AND METHODS). To determine which meiotic division had taken place, we examined the dyads that had two viable diploid spores (89 out of 97). Such dyads held all the meiotic products of a given meiosis and therefore contained complete information concerning it. We expected these dyads to have resulted from cells which had either gone through the first or the second meiotic division (the mating type locus is 25 cM away from the centromere of chromosome *III* (MORTIMER and SCHILD 1980), allowing the formation of **a**/α diploid dyads in either case). A diploid strain which is heterozygous for centromere-linked markers is expected to give rise to homoallelic spores following a first meiotic division, or alternatively, to heteroallelic spores following a second meiotic division. We followed the segregation patterns of markers on four chromosomes. The expected segregation patterns of centromere-linked markers are shown in Figure 1, a and b. Surprisingly, many of the diploid dyads exhibited a mixed-segregation phenotype. Some of the centromere-linked markers underwent first division segregation whereas others, in the same cell, underwent second division segregation (Figure lc). Similar results were obtained when the cells were sporulated at semirestrictive tem-

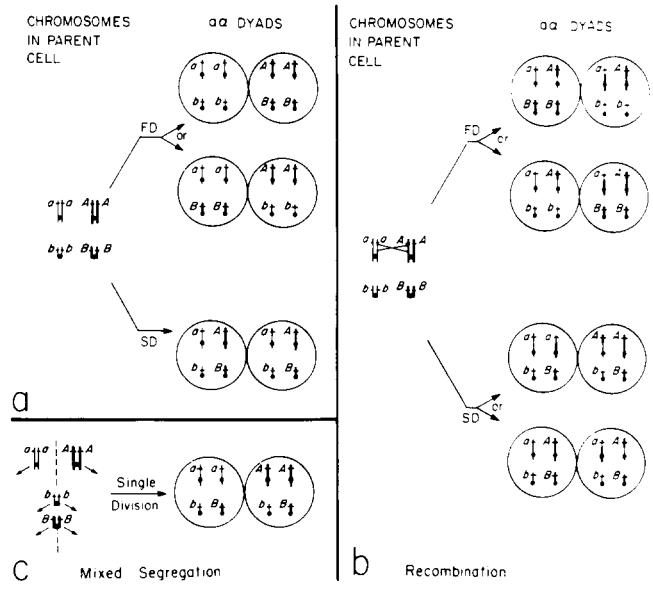

FIGURE 1.—Products of single-division meiosis. Homologous chromosomes are drawn similar in length and shape but different in width. *A* and *B* indicate dominant alleles of centromere-linked markers on different chomosomes. *a* and *b* indicate the respective recessive alleles. Black circles indicate centromeres. (a) Simple segregations in a single division: top, two alternatives of FD (first division) segregation; bottom, SD (second division) segregation. (b) A single crossover event between marker A and the centromere followed by either FD or SD segregation. (c) Autonomous segregation of independent chromosomes; one pair of chromosomes undergoing FD segregation and another going through SD segregation (within the same cell).

TABLE 3

Detailed segregations of centromere-linked markers in four dyads carrying two viable diploid a/α spores

Dyad no.	Spore	Genotype									
		MAT	*ADE1*	*TRP1*	:	*CDC7*	*LEU1*	:	*URA3*	*MET14*	
16	a	a/α	+/−	+/−		−/+	−/−		+/+	−/−	
	b	a/α	+/−	+/−		−/+	+/+		−/−	+/+	
Segregation			2	2	:	2	1	:	1	1	
5	a	a/α	+/−	+/+		+/+	−/−		+/+	−/−	
	b	a/α	+/+	+/−		−/+	+/+		−/−	+/+	
Segregation			*	p		*	1	:	1	1	
60	a	a/α	+/+	+/−		−/	+/−		+/+	−/	
	b	a/α	−/−	+			+/−		−/−	−	
				+/−		−/				+/+	
				+							
Segregation			1	2	:	2	2	r	1	1	
82	a	a/α	+/+	+/−		?	+/−		+/−	+/−	
	b	a/α	−/−	−/−		?	+/−		+/−	+/−	
Segregation			1	*		?	2	dr	2	2	

A detailed presentation of four of 89 dyads which had two viable **a**/α diploid spores (see Table 4). 1, 1st meiotic segregation; 2, 2nd meiotic segregation; :, linked genes; r, single recombination between linked genes; dr, double recombination between linked genes; *, gene conversion; p, (pms) postmeiotic segregation (in this case spore b gave rise to a culture with two types of tetrads with respect to *TRP1* segregation, 2+:2− and 0+:4−; spore a gave rise to 4+:0− tetrads and therefore the projected original segregation was 5:3); ?, not checked.

perature (G. SHARON, unpublished data). Four examples of the phenotype, some of which are quite rare, are shown in Table 3 to illustrate the method of analysis. A summary of the data from 89 dyads is shown in Table 4. The results demonstrate that most dyads (54 out of 89) exhibit a mixed-segregation phenotype. Table 5 shows the segregation patterns of markers in **a**/α spores from dyads with a single viable **a**/α spore (105 such spores, see Table 2), or dyads with two viable spores, one of which is **a**/α and the other either **a**, α, or undetermined (48 such spores, see Table 2). Again, most of these spores exhibit mixed segregation patterns rather than a 1st meiotic segregation pattern (class no. 1), or a 2nd meiotic segregation pattern (class no. 16).

Recombination does not explain mixed segregation patterns: The mixed segregation of the markers could reflect a genuine heterogeneity in the segregation patterns of chromosomes, or could result from exceptionally high recombination rates between the centromeres and the various markers (see Figure 1b). In order to obtain recombination frequencies in the centromeric regions and follow the segregation patterns of chromosomes, we examined chromosomes bearing pairs of centromere-linked markers, on both sides of the same centromere. The recombination frequency between a pair of centromere-bracketing markers in the diploid dyads could be compared to the frequency in tetrads. An additional bonus of such a design is that the segregation pattern of a given centromere can be determined with a higher certainty by following the segregation of the markers on both sides of the centromere.

Chromosomes *IV* of strain 1122 are heterozygous *in trans* for *trp1* (which is located on the right arm, about 2.3 cM from *CEN4*, see Table 6) and *cdc7* (which is located on the opposite arm, 4.4 cM distal from *CEN4*, see Table 6). Thus the genotypes at both loci, *TRP1* and *CDC7*, were used to follow the segregation of *CEN4*. In order to follow the segregation of centromere-bracketing markers on another pair of chromosomes, we integrated a *URA3* gene into the right arm of one of the chromosome *VII* homologs, 7.7 cM away from *CEN7* (*leu1* is located on the left arm of the same chromosome, 4.1 cM from *CEN7*, see Table 6). The strain in which *URA3* is integrated near *CEN7* is called 1122-1U, and was used for all of the experiments reported here.

In the dyads, the recombination distances between *TRP1* and *CDC7* and between *LEU1* and the integrated *URA3* were 1.9 and 13.6 cM, respectively. In tetrads the distances were 5.9 and 15.4 cM, respectively (see Table 6). The differences between dyads and tetrads may be due to sample size. At any rate, high frequency of recombination around the centromeres as the cause of the mixed segregation phenotype, can be ruled out.

In a *bona fide* reductional-division segregation for a chromosome which is marked by centromere-bracketing markers, both spores should be homoallelic for the markers. In equational division segregations, both spores should be heteroallelic for both markers. If the alleles of the two marker genes are situated *in trans* on the two homologous chromosomes, each of the four chromatids involved in either type of segregation should bear a dominant allele of one of the markers

TABLE 4

Diploid dyads with two viable a/α spores: segregation of centromere-linked markers

Chromosomes:	*I*	*IV*	*VII*	*XI*		*I*	*IV*	*VII*	*XI*		*I*	*IV*	*VII*	*XI*
Markers:	A	T:C	L:U	M		A	T:C	L:U	M		A	T:C	L:U	M
1)	2	1−1	2 *	1 dis	31)	1	2−2	2−2	2	60)	1	2−2	2+1	1 dis
2)	2	2−2	2−2	1	32)	1	2−2	1−1	2	61)	1	2 ?	2+1	1
3)	1	2−2	2−2	1 dis	33)	1	1−1	1−1	1 dis	62)	1	2−2	1−1	1
4)	*	2−2	2−2	*	34)	*	1−1	1−1	1	63)	2	2−2	2−2	*
5)	*	p *	1−1	1 dis	35)	2	1+2	1 *	1	64)	*	1−1	1−1	1 dis
6)	2	1+2	2−2	1	36)	*	1−1	1−1	1	65)	*	2−2	2+2	*
7)	*	1−1	2−2	1 dis	37)	1	1−1	1−1	1	66)	1	1−1	1−1	1
8)	1	2−2	2−2	1 dis	38)	2	1−1	* *	1 dis	67)	1	2−2	1−1	2 dis
9)	2	2−2	1+2	2	39)	2	1−1	1−1	1	68)	*	2−2	* 1	1 dis
10)	*	2−2	1+2	2 dis	40)	1	1−1	1−1	1	69)	1	2−2	1+2	1 dis
11)	1	1−1	2+1	2	41)	2	2−2	1−1	1	70)	1	1−1	1−1	1
12)	*	2−2	2−2	1	42)	1	2−2	* 2	1	71)	2	1−1	2−2	1 dis
13)	*	1−1	1−1	1	43)	*	2−2	2+1	1	72)	1	1−1	1−1	1
14)	1	1+2	2+2	* dis	44)	1	1−1	1−1	1	73)	2	1−1	2−2	1
15)	*	2−2	1−1	1	45)	1	1−1	1−1	1	74)	1	2 ?	1+2	1 dis
16)	2	2−2	1−1	1	46)	2	2−2	1−1	2 dis	75)	1	2−2	1−1	*
17)	*	2−2	1−1	2	47)	2	2−2	1−1	1	76)	1	2−2	2−2	1 dis
18)	1	1−1	1−1	1	48)	1	1 *	1−1	1	77)	1	2−2	1+2	1
19)	1	1−1	1−1	1	49)	1	1−1	1−1	1	78)	1	1−1	1−1	1
20)	2	* 2	1−1	2 dis	50)	*	2−2	1−1	2	79)	*	2−2	1−1	1 dis
21)	1	1−1	* 2	2	51)	*	1−1	2−2	* dis	80)	1	2−2	2−2	1
22)	1	2−2	2−2	1	52)	1	1−1	1−1	1	81)	*	1−1	1−1	1
23)	*	* 2	2−2	2	53)	2	1−1	2+1	* dis	82)	1	* ?	2×2	2
24)	p	p 2	1−1	1 dis	54)	*	1−1	1−1	1 dis	83)	2	2−2	* 2	2
25)	*	1−1	1−1	*	55)	2	2−2	2−2	1	84)	2	2−2	2−2	2
26)	*	2−2	2+2	1	56)	1	2−2	2−2	2	85)	*	1−1	1−1	1
27)	2	2−2	1−1	1	57)	2	2−2	2−2	2	86)	*	* *	1−1	1
28)	1	2−2	2−2	2	58)	2	1−1	1+2	1	87)	1	2−2	2−2	1 dis
29)	1	1 ?	1+2	1	59)	1	1−1	1+2	1	88)	2	1−1	1−1	1
30)	*	* ?	1 *	1 dis						89)	2	2−2	1−1	1 dis

Summary: segregation frequencies of each of the markers (gene conversion cases are excluded)

	ADE1	*TRP1*	*CDC7*	*LEU1*	*URA3*	*MET14*
Equational segregation/total	24/63	43/82	47/81	32/84	33/86	18/81
	38.1%	52.4%	58.0%	38.1%	44.2%	22.2%

1, 1st meiotic division segregation; 2, 2nd meiotic division segregation; A, T, C, L, U, M, the markers *ADE1, TRP1, CDC7, LEU1, URA3, MET14*, respectively; p, (pms) postmeiotic segregation; *, gene conversion; dis, genotype checked by dissection of tetrads of the diploid colonies formed by each of the dyad's spores; ?, genotype was not checked; :, linked markers; −, +, ×, recombination between two linked markers: no crossing over, single, and double crossovers, respectively (corresponding to PD, TT, and NPD in tetrads, respectively).

and a recessive allele of the other. In diploid dyads, a reductional division segregation would result in one spore being +− and the other being −+ for the phenotypes of the two markers (and +−/+− and −+/−+, respectively, for the genotypes). An equational division would result in the two spores being of phenotype ++ (and genotype +−/−+). All single crossover events between the markers and most of the possible double crossover events would result in chromatids marked *in cis* and thus be identified.

Double crossover events, in which only two of the four chromatids were involved, appear as noncrossover cases. Such cases in which the first crossover was between the centromere and one marker, and the second was between the centromere and the other marker, could be misleading because reductional segregations in these cases would seem as equational and one half of the equational segregations would seem as reductional ones. Two-strand double crossovers constitute 0.25 of the possible double crossover cases. Considering the genetic distances of the markers from their respective centromeres, and assuming no interference, the expected frequency of double crossovers of the relevant types is 0.101% (0.046 × 0.088 × 0.25) for chromosomes *IV*, and 0.316% (0.082 × 0.154 × 0.25) for chromosomes *VII*. The frequency of cases in which one pair of chromosomes could have erroneously seemed to have segregated differently from the other pair due to double crossover is therefore expected to be 0.417% (0.023% + 0.164%). The actual frequency of such segregations was 25% (18/71, see Table 4; only dyads, in which the segregation of all four markers had been determined, were scored), a value 60-fold greater than the expected one. Furthermore, the frequency of dyads with *detected* single crossovers between the markers of either chro-

TABLE 5

Segregation of the four centromere-linked markers in dyads where only one of the spores could be scored (16 possible classes)

Class	Markers				Dyad type		
	ADE	*TRP*	*LEU*	*MET*	A	B	C
1)	1	1	1	1	27	11	5
2)	1	1	1	2	2	1	
3)	1	1	2	1	7	1	
4)	1	2	1	1	8		2
5)	2	1	1	1	12	1	
6)	1	1	2	2	4		2
7)	1	2	2	1	4	3	1
8)	2	2	1	1	7	2	
9)	1	2	1	2	2		2
10)	2	1	2	1	2	1	
11)	2	1	1	2	0		
12)	2	2	2	1	10	3	4
13)	2	2	1	2	5		
14)	2	1	2	2	2		
15)	1	2	2	2	8	2	1
16)	2	2	2	2	5	2	4
				Total	105	27	21

1, 1st meiotic segregation; 2, 2nd meiotic segregation; A, dyads having a single viable spore; B, dyads having one **a**/α spore and another spore which is either an **a**-mater or an α-mater; C, dyads having one **a**/α spore and another, undertermined spore (a non-mater which is unable to sporulate).

TABLE 6

Map distances of the genetic markers

Chromosome no.	Interval	Segregation (no.)		Ascus type (no.)			Map distance (in cM)
		FD	SD	PD	NPD	T	
A							
I	*ADE1-CEN1*	148	23				6.7
IV	*TRP1-CEN4*	163	8				2.3
IV	*CEN4-CDC7*	31	3				4.4
VII	*LEU1-CEN7*	159	13				4.1
VII	*CEN7*-int*URA3*	66	12				7.7
XI	*MET14-CEN11*	169	2				0.6
B							
IV	*TRP1-CDC7*			30	0	4	5.9
VII	*LEU1*-int*URA3*			59	1	18	15.4
C							
IV	*TRP1-CDC7*			76	0	3	1.9
VII	*LEU1*-int*URA3*			64	1	16	13.6

Map distances between markers in strain 1122 and derivative strains. FD, first division segregation; SD, second division segregation; PD, parental ditype; NPD, nonparental ditype; T, tetratype; A, map distances of the markers from their respective centromeres (tetrad analysis); B, map distances of linked markers (tetrad analysis); C, map distances of the same markers in dyads (as calculated from the data in Table 4); int*URA3*, a *URA3* marker integrated at a centromere-linked site in the right arm of chromosome *VII*.

mosome pair was 21% (15/71, see Table 4). Had the mixed segregation phenotype been the outcome of double crossing over, its frequency should have been much lower than the frequency of detected single crossover events (assuming that the frequency of double crossovers equals the product of probabilities of single crossovers). We conclude that the differences between the segregation patterns of the centromere-bracketing markers of these two chromosomes (*IV* and *VII*), and most likely also the patterns of the centromere-linked markers of the other two chromosomes (*I* and *XI*), are not due to increased recombination between the markers and the centromeres. Rather, these segregation patterns must have resulted from a mixed segregation of chromosomes, namely some of the chromosomes have segregated reductionally, whereas others, within the same cells, have segregated equationally.

No aberrant segregations were observed among the diploid dyads: By analyzing colonies of 89 **a**/α diploid dyads with two viable spores, we followed the segregation of 529 cases of centromere-linked markers (89 × 6 markers − 5 cases in which the segregation of *cdc7* was not checked, see Table 4). In 10% of the segregations (53 cases), one diploid colony seemed heterozygous, whereas the other seemed homozygous for a given marker. Such a phenomenon could have resulted from one of three possible events: (1) gene conversion, (2) nondisjunction and (3) chromosome loss. Gene conversion would give rise to two normal diploid spores, one heterozygous and one homozygous for the converted marker. The outcome of nondisjunction would be one spore which was trisomic and another which was monosomic for the given chromosome. As a result of chromosome loss (after separation of the chromatids), one of the spores would be a normal diploid and the other monosomic for the given chromosome. In order to distinguish between these three possibilities, tetrad analysis had to be performed on asci produced by these **a**/α diploid colonies (random spore analysis could not distinguish a heterozygous diploid from a trisomic colony or a homozygous diploid from a monosomic one). Tetrads produced by a homozygous diploid were expected to give rise to four viable progeny each. On the other hand, tetrads produced by a monosomic colony were expected to have two viable and two nonviable sister spores. Tetrad analysis of diploid colonies from both spores of a dyad was therefore performed in 23 of these cases (the tetrads marked "dis"–for dissection–in Table 4). All the colonies that were analyzed proved to be normal **a**/α diploids. It appears that although the segregation was "mixed" at the cellular level, at the level of individual chromosomes the segregation was regular and nonperturbed. No aberrant segregations were detected. Each chromosome pair had segregated either reductionally or equationally. The frequencies of gene conversion and postmeiotic segregation were 29.2% for *ADE1*, 7.1% for *TRP1*, 3.6% for *LEU1* and 9.0% for *MET14* (Table 4). These frequencies are high in comparison to the ones reported in the literature (Fogel *et al.* 1978; Nicolas *et al.* 1989), and

TABLE 7

Genotype of dyads having one diploid a/α spore and another which is either a or α

Dyad spore	Markers				
	MAT	ADE1	TRP1	LEU1 : URA3	MET14
1 a	**a**	+	−	+ −	+
b	**a**/α	−/−	+/+	−/− +/+	−/−
2 a	**a**/α	−/−	−/−	+/+ −/−	−/−
b	**a**	+	+	− +	+
6 a	**a**	+	−	− +	+
b	**a**/α	−/−	+/+	+/+ −/−	−/−
7 a	**a**/α	−/−	+/−	+/− r −/−	+/−
b	(α/α)	probably diploid			
9 a	**a**/α	+/−	+/−	+/+ r +/	+/+
b	α	+	+	−	−
14 a	(**a**/**a**)	probably diploid			
b	**a**/α	+/−	+/+	+/− −/+	+/−
15 a	**a**/α	+/+	−/−	+/+ −/−	+/+
b	**a**	−	+	− +	−
16 a	(α/α)	probably diploid			
b	**a**/α	+/−	+/+	−/− +/+	−/−
19 a	(α/α)	probably diploid			
b	**a**/α	+/+	+/−	+/− −/+	−/−
21 a	**a**/α	−/−	+/−	+/− r +/+	+/−
b	(α/α)	probably diploid			
24 a	**a**/α	+/−	+/−	+/− −/+	+/+
b	α	−	+	+ −	+*
26 a	**a**/α	+/+	+/−	+/− −/+	+/+
b	**a**	−	−	+ −	+*

*, Gene conversion; :, linked genes; r, recombination between linked genes.

are consistent with previous data, showing high meiotic gene conversion rates in *cdc5/cdc5* diploids (SIMCHEN *et al.* 1981).

Dyads with a diploid a/α spore and a haploid spore: In 27 of 614 dyads with two viable spores, the colony formed from one spore was a nonmater that could sporulate and the colony from the other spore was either an **a** or an α-mater. Twelve of these dyads were analyzed genetically (Table 7). All the nonmating colonies were shown by marker segregation in tetrad analysis to be **a**/α diploids.

In order to determine the genotype of the mater colonies we mated them to haploid tester strains. Each colony was mated to two such testers, one prototrophic and the other auxotrophic for the centromere-linked markers, and the sporulation products of each cross were analyzed. Tetrads from crosses to seven of the colonies had four viable spores, and segregation of the markers was as expected of a diploid. The original colonies were therefore haploids. Thus in the original 7 (out of 12) dyads, one spore was an **a**/α diploid and the other a mater haploid (either **a** or α). Meioses in which diploid and haploid spores were produced within the same ascus have been reported previously (THOMAS and BOTSTEIN 1986). Those asci were interpreted to have resulted from a meiosis in which only one of the two first-division products had gone through a second (equational) division. In our analysis, four of the seven diploid-haploid dyads (nos. 1, 2, 6 and 15 in Table 7) could fit into that category (because the diploid appears to be the product of a reductional division). An additional haploid product in each ascus might have degenerated during the sporulation process. Colonies formed by the diploid spores of the other three dyads (nos. 9a, 24a and 26a) exhibited a mixed segregation of chromosomes, or possibly, in dyad no. 26, even an equational segregation (because of a gene-conversion event at *MET14*). Note, that the latter case might also have been a mixed segregation that was not detected as such, because segregation patterns of only three out of 16 chromosomes were determined.

Crosses of the other five mater colonies, gave rise to strains with very poor spore viability and most likely resulted from triploid segregations, the triploid having been generated by the mating of a diploid mater segregant to the haploid tester. If so, both spores of these dyads were diploid, with one of the *MAT* alleles having undergone gene conversion.

Chromosome pairs differ in segregation tenden-

cies: Although there was no rigid rule as to which of the chromosomes underwent a reductional or an equational segregation in any single event of mixed meiotic segregation, a summary of many events showed significant differences between the four chromosomes (Table 4). Chromosome *IV* (marked by *TRP1*), for example, had segregated equationally in 52% of the examined cases whereas chromosome *XI* (marked by *MET14*), had segregated equationally only in 22% of these meioses. The differences between the segregation frequencies of the four chromosomes were highly significant ($\chi^2_{[3]} = 15.85$) (SNEDECOR and COCHRAN 1967). The pairwise comparisons of segregation frequencies between chromosomes *I* and *XI*, *IV* and *XI*, *VII* and *XI* showed significant differences. The difference *I–VII* was, of course, not significant, whereas the *I–IV* and *IV–VII* comparisons were of borderline significance.

Effects of lethality and gene conversion on segregation tendencies: Since spore viability was not high (Table 2), it seemed possible that differential lethality affected the results. Selection against certain segregation products could account for the observed differences in segregation frequencies. In order to test this possibility, we have compared chromosome segregation patterns between dyads of two categories, those having two viable spores and those having only one viable spore per dyad. In case of selection, dyads of the first category were expected to have reduced frequencies of certain genotypes. Dyads of the second category on the other hand, were expected to have increased frequencies of these same genotypes (because lethality of a spore in a given dyad would withdraw the dyad from the first category and place it in the second one).

Comparison between dyads with two viable spores and those with one viable spore was carried out after necessary adjustments were made (see MATERIALS AND METHODS). The adjustments were required because, in some cases, information about each meiosis was obtained from analysis of two spores, whereas in others it was obtained from analysis of only one spore. The comparison was carried out as follows. Dyads of the two categories were distributed between different segregation classes (according to the way the markers had segregated), and the numbers of dyads or spores in the different classes were compared. Due to the limited size of the two categories, we performed four tests analyzing three markers in each instead of performing one test analyzing four markers (in every test we ignored a different marker). By doing so, we followed 8 (2^3) possible segregation classes in every test (instead of 16, see Table 8), and the number of expected cases in each class was sufficient to be tested statistically. The comparisons showed that the segregation differences between two-spore dyads and "one-spore" dyads were not significant (Table 8) and spore lethality may be considered random. The differences in segregation frequencies must therefore reflect a dissimilarity in the segregation tendencies of different chromosomes at the given conditions.

We also asked whether the relatively high rate of gene conversion had an effect on the segregation tendencies of the marked chromosomes. We performed a similar comparison in which all dyads with two viable spores having gene-conversions or post-meiotic segregations were excluded. The differences were still not significant (Table 8). The effects of gene conversion and post-meiotic segregation are therefore negligible.

Mixed meiotic segregation in *cdc14* homozygotes: *cdc14* homozygotes were also sporulated under shift-down conditions as described above. Analysis of 62 dyads of strain 3344 (marked by *ADE1*, *TRP1*, *LEU1* and *MET14*, see MATERIALS AND METHODS), showed 43 to have undergone mixed meiotic segregation for the marked centromeres. The tendencies of the four marked chromosomes to segregate equationally were 42% for *ADE1*, 58% for *TRP1*, 29% for *LEU1* and 27.4% for *MET14*. These relative tendencies are similar to the ones observed for *cdc5* homozygotes. We conclude that the mixed segregation phenotype is not unique to *cdc5* mutants.

DISCUSSION

Meiosis usually consists of two divisions which follow a single replication of the genome, resulting in a reduction in the number of chromosomes per nucleus. Every diploid cell undergoing meiosis gives rise to four haploid nuclei. The main difference between the first and the second divisions of meiosis is that, in the first, the two homologous chromosomes segregate from each other while the sister-centromeres are held together and migrate to the same pole, whereas in the second division, the sister-centromeres segregate from each other, pulling the chromatids to opposite poles. When only a single division occurs in meiosis instead of the usual two, two products are obtained which are diploid rather than haploid. Such dyads (two-spored asci) with diploid spores were observed in meiosis of yeast mutants in which only the first (SCHILD and BYERS 1980) or the second meiotic division (KLAPHOLZ and ESPOSITO 1980) took place. The determination as to which of the two divisions took place was based on the segregation of centromere-linked markers. In the case we present here, most of the diploid spores resulted from single divisions in which some centromeres segregated reductionally, whereas others, in the same cell, segregated equationally.

No cases of chromosome loss or nondisjunction were detected among the dyads observed. It is thus unlikely that the mixed segregation phenotypes re-

TABLE 8

Distribution of segregants: comparison between dyads having two viable (viab.) spores (sp.) and dyads with one viable spore

A					B				
Markers			2 Spores viable	1 Spore viable	Markers			2 Spores viable	1 Spore viable
ADE	*TRP*	*MET*			*ADE*	*TRP*	*LEU*		
1	1	1	30.5	34	1	1	1	29.0	30
1	1	2	5.0	6	1	1	2	5.5	11
1	2	1	21.5	12	1	2	1	13.5	10
2	1	1	15.5	14	2	1	1	10.0	12
2	2	1	16.5	17	2	2	1	13.0	12
2	1	2	2.0	2	2	1	2	7.5	4
1	2	2	11.0	10	1	2	2	19.0	12
2	2	2	11.0	10	2	2	2	14.5	15
Total			113	105	Total			113	105

C					D				
Markers			2 Spores viable	1 Spore viable	Markers			2 Spores Viable	1 Spore Viable
TRP	*LEU*	*MET*			*ADE*	*LEU*	*MET*		
1	1	1	38.0	39	1	1	1	37.5	35
1	1	2	2.0	2	1	1	2	6.0	4
1	2	1	8.0	9	1	2	1	14.5	11
2	1	1	17.0	15	2	1	1	17.5	19
2	2	1	21.0	14	2	2	1	14.5	12
2	1	2	9.5	7	2	1	2	5.5	5
1	2	2	5.0	6	1	2	2	10.0	12
2	2	2	12.5	13	2	2	2	7.5	7
Total			113	105	Total			113	105

χ^2 values obtained : $\chi^2_A = 3.22$ $\chi^2_B = 4.57$ $\chi^2_C = 1.99$ $\chi^2_D = 1.06$

χ^2 values after exclusion of cases with gene conversion : $\chi^2_A' = 5.33$ $\chi^2_B' = 9.64$ $\chi^2_C' = 5.18$ $\chi^2_D' = 1.39$

A–D, four comparison tests. A total of 137 dyads with two viable spores were analyzed: 89 of them had two **a**/α diploid spores and 48 had only one **a**/α spore. Due to comparison considerations, the latter spores were calculated as "half cases" (see MATERIALS AND METHODS). The total number of two-viable-spores dyads was therefore calculated as 113. A total of 105 dyads with one viable spore were analyzed. In each test, the segregation of three of the four markers is shown (in each table the segregation of a different marker is ignored). 1, 1st division segregation; 2, 2nd division segregation. The eight possible segregation-categories are shown at the left side of each test. The right and middle columns of each test show the distribution of dyads having one viable and two viable spores, respectively, among the different segregation categories. A chi-square test was performed for each table (SNEDECOR and COCHRAN 1967), χ^2_A–χ^2_D. The second set of chi-square values, χ^2_A'–χ^2_D', was obtained after exclusion of cases where one or more of the relevant markers had undergone gene-conversion or postmeiotic segregation (the second set of tables is not shown, only the final results are given). The value of χ^2 for $P \leq 0.05$ (7 d.f.) is 14.07. This value is much higher than any of the values obtained.

sulted from precocious separation of sister chromatids, followed by independent distribution between the two diploid spores, as this would produce a high frequency of aberrant segregations. Our data indicate therefore that every chromosome pair in those dyads underwent a genuine segregation, either reductional or equational. We term the phenomenon of mixed reductional and equational chromosome segregation "mixed meiotic segregation."

In normal meiosis all the chromosomes segregate reductionally in the first division and equationally in the second, pointing to the existence of a mechanism that controls the type of segregation at the cellular level. From the occurrence of "mixed-meiotic segregation," we infer that chromosome segregation must also be regulated individually for each pair of homologous chromosomes. Furthermore, each chromosome pair has a unique segregation tendency. Chromosomes *IV* tend to undergo reductional segregation, whereas the homologs of chromosome *XI* tend to go through equational segregation. Chromosomes *I* and *VII* have intermediate segregation patterns.

The differences between these tendencies cannot be explained by high rates of nondisjunction or chromosome loss, by increased recombination near the centromeres, or by gene conversion, for the following reasons:

1. Neither nondisjunction nor chromosome loss was detected in any of the dyads examined.

2. Increased recombination near the centromeres was ruled out experimentally for chromosome pairs *IV* and *VII* (see Table 3). The high tendency of chromosome *XI* to segregate reductionally cannot be due to recombination. High recombination rates between *MET14* and *CEN11* would have modified the real values and brought them nearer to the intermediate

tendencies, such as the ones recorded for chromosome pairs *I* or *VII*.

3. Statistical analysis has shown that the effects of gene conversion on segregation tendencies were negligible (although the frequency of gene conversion events was quite high).

The unique segregation tendencies of different chromosomes show that some factor, specific for each chromosome pair, influences the type of segregation they go through. Chromosome length does not seem to be this factor. Chromosome *IV* is the longest yeast chromosome, whereas chromosome *I* is the shortest. Chromosome *XI*, with the highest tendency to segregate reductionally, has an intermediate length (CARLE and OLSON 1985). Experiments reported in a companion study (SHARON and SIMCHEN 1990) demonstrate that these putative chromosome-specific factors are located near, or at, the centromeres of the given chromosomes.

The roles of the *CDC5* and *CDC14* gene products in nuclear division are unknown. They are required for both mitosis and meiosis because *cdc5* and *cdc14* mutants arrest at high temperatures in mitosis as well as in the first and second divisions of meiosis (SCHILD and BYERS 1980; G. SHARON, unpublished results). One possibility is that the *CDC5* and *CDC14* products take part in the interaction between the centromeres and the spindle fibers, an interaction which is responsible for the segregation of each chromosome pair. However, it is possible that the unique chromosome behavior is not directly related to the specific *cdc5* or *cdc14* defects, but is rather related to the general transient arrest or slowing down of meiosis in these mutants.

The mixed meiotic segregation behavior is not unique to *cdc5* or *cdc14* mutants. It has also been observed among *spo12* and *spo13* dyads (KLAPHOLZ and ESPOSITO 1980), which usually but not always result from failure of the first meiotic division. Furthermore, haploid *spo13* strains disomic for chromosome *III* and going through "haploid meiosis," show a high frequency (around 40%) of the equivalent of "mixed meiotic segregation," namely the chromosomes *III* segregate reductionally, whereas the other chromosomes divide equationally (WAGSTAFF, KLAPHOLZ and ESPOSITO 1982; HOLLINGSWORTH and BYERS 1989). Among the haploid-meiosis products, many (10–30%) were monosomic or trisomic for chromosome *III*, suggesting a general inaccurate disjunction of chromosomes. In this respect meiosis in *spo13* mutants appears to be more aberrant than the single-division segregations reported here for *cdc5* homozygotes.

Since no ultrastructural analysis of "mixed segregation" has been done so far, we do not have a definite picture as to how many spindle poles are involved in the phenomenon. It could either be two or four spindle poles. In the former case, different bivalents are oriented in two different ways on one spindle. In the latter case, four spindle poles are present in a common nucleus (as was the phenotype of late arrested cells, observed by SCHILD and BYERS 1980). Although we cannot discard the latter possibility, we favor the former for the following reasons: (1) At the time of the release from the temperature block, the arrested mononucleated cells had one spindle. Since cells were permitted to complete the meiotic process, there is no reason to assume that they were arrested at a second arrest point. (2) The detection of some cells which were able to undergo an additional division following the "mixed" one (dyads 9, 26 and possibly 24 in Table 7), suggests the existence of only one spindle at the stage of the "mixed" segregation. It also suggests that the reductional and equational segregation of the different chromosomes occur simultaneously. However, since this observation is based on a very small number of cases, it should be regarded cautiously. It should also be noted that in these dyads, an equational segregation of nine of the chromosomes was followed by another segregation. Pairs that have segregated equationally during the first meiotic division are expected to have difficulties in segregating again: homologs can not separate efficiently from one another without a first-division machinery. A high rate of aberrant segregation was therefore expected among these chromosomes, but none was found.

By micromanipulation, NICKLAS (1977) has transferred chromosomes undergoing meiosis in male grasshoppers from one spindle to another. He reported that first-division bivalents placed in a second-division spindle continued their reductional separation, whereas second-division chromosomes in a first-division spindle continued to divide equationally. This finding further supports the interpretation of single-division meiosis given above, namely that on a single spindle apparatus, some chromosomes segregate reductionally while others do so equationally.

We thank DAVID GRANOT for suggesting the possibility of "mixed meiotic segregation," SHOSHANA KLEIN and YONA KASSIR, for valuable comments on the manuscript, and JEANNE P. MARGOLSKEE for plasmids. This research was supported by grant 84–00231 from the United States-Israel Binational Science Foundation (BSF), and by grant I-59–283.3/87 from the German Israeli Foundation for Scientific Research and Development (GIF).

LITERATURE CITED

CARLE, G. F., and M. V. OLSON, 1985 An electrophoretic karyotype of yeast. Proc. Natl. Acad. Sci. USA **82:** 3756–3760.

DAVIDOW, L. S., L. GOETSCH and B. BYERS, 1980 Preferential occurrence of non-sister spores in two-spored asci of *Saccharomyces cerevisiae*: evidence for regulation of spore-wall formation by the spindle pole body. Genetics **94:** 581–595.

FOGEL, S., R. MORTIMER, K. LUSNAK and F. TAVERES, 1978 Meiotic gene conversion: a signal of basic recombination

in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:** 1325–1341.

HICKS, J. B., and I. HERSKOWITZ, 1976 Interconversion of yeast mating types. I. Direct observations of the action of the homothalism (HO) gene. Genetics **83:** 245–258.

HOLLINGSWORTH, N. M., AND B. BYERS, 1989 *HOP1*: a yeast meiotic pairing gene. Genetics **121:** 445–462.

KASSIR, Y., and G. SIMCHEN, 1985 Mutations leading to expression of the cryptic *HMRa* locus in the yeast *Saccharomyces cerevisiae*. Genetics **109:** 481–492.

KLAPHOLZ, S., and R. E. ESPOSITO, 1980 Recombination and segregation during the single-division meiosis in *spo12–1* and *spo13–1* diploids. Genetics **96:** 589–611.

MORTIMER R. K., and D. SCHILD, 1980 Genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44:** 519–571.

NICKLAS, R. B., 1977 Chromosome distribution: experiments on cell hybrids and *in vitro*. Philos. Trans. R. Soc. Lond. **277:** 267–276.

NICOLAS, A., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. Nature **338:** 35–39.

ORR-WEAVER, T. E., J. W. SZOSTAK and R. J. ROTHSTEIN, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

ROTHSTEIN R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SCHERER, S., and R. W. DAVIS, 1979 Replacement of chromosome segments with altered DNA sequence constructed *in vitro*. Proc. Natl. Acad. Sci. USA **76:** 4951–4955.

SCHILD, D., and B. BYERS, 1980 Diploid spore formation and other meiotic effects of two cell division cycle mutations of *Saccharomyces cerevisiae*. Genetics **96:** 859–876.

SHARON, G., and G. SIMCHEN, 1990 Centromeric regions control autonomous segregation tendencies in single-division meiosis of *Saccharomyces cerevisiae*. Genetics **125:** 487–494.

SHILO, V., G. SIMCHEN and B. SHILO, 1978 Initiation of meiosis in cell cycle initiation mutants of *Saccharomyces cerevisiae*. Expt. Cell Res. **112:** 241–248.

SIMCHEN, G., R. PINON and Y. SALTS, 1972 Sporulation in *Saccharomyces cerevisiae*: premeiotic DNA synthesis, readiness and commitment. Expt. Cell. Res. **75:** 207–218.

SIMCHEN, G., Y. KASSIR, O. HORESH-CABBILLY and A. FRIEDMANN, 1981 Elevated recombination and pairing structures during meiotic arrest in yeast of the nuclear-division mutant *cdc5*. Mol. Gen. Genet. **184:** 46–51.

SNEDECOR, G. W., and W. G. COCHRAN, 1967 *Statistical Methods*, Ed. 6. Iowa State University Press, Ames, Ia.

THOMAS, J. H., and D. BOTSTEIN, 1986 A gene required for the separation of chromosomes in the spindle apparatus in yeast. Cell **44:** 65–76.

WAGSTAFF, J. E., S. KLAPHOLZ and R. E. ESPOSITO, 1982 Meiosis in haploid yeast. Proc. Natl. Acad. Sci. USA **79:** 2986–2990.

Communicating editor: G. S. ROEDER